SOLUTION FOR DIAGNOSING OR TREATING TISSUE PATHOLOGIES

Technical Realm

The present invention concerns a 5-aminolevulinic acid ester (E-ALA) for producing a pharmaceutical preparation used in the diagnosis and treatment of tissue and/or cellular pathologies by local radiation exposure using radiation emitted by a light source followed, in the case of diagnosis, by detection of fluorescence emitted by the substances for which the 5-aminolevulinic acid ester (ALA) or the E-ALA are precursors, particularly protoporphyrin IX (PpIX).

Prior Art

The use of compounds for which ALA or ALA esters (E-ALA) and particularly hexylester hydrochloric ALA (h-ALA) are precursors is well known in the diagnosis and/or treatment of lesions, particularly cancerous lesions. This principle is thoroughly discussed in patent Publication No. WO 96/28412. The solution may be administered orally or parenterally, for example, by intra-dermal, subcutaneous, intra-peritoneal or intravenous injection. It may also be administered topically, for example locally, by exposing the surface of the organ to be treated to an E-ALA or ALA solution. A pad saturated with such a solution can also be used during topical administration. The concentration of the ALA (E-ALA) ester solution mentioned in this publication ranges from 1 to 50% and preferably between 15% and 30%. However, this concentration generates essentially no PpIX in certain organs which are principally involved in this type of treatment, namely the bladder.

In the publications in the *Journal of Photochemistry and Photobiology B* and *Biology* , respectively, by Fin-Puches et al entitled "Primary Clinical Response and Long-Term Follow-Up of Solar Keratoses Treated with Topically Applied 5-Aminolevulinic Acid and Irradiation by Different Wave Bands of Light," and by Chang et al entitled "The Efficacy of an Iron Chelator (CP94) in Increasing Cellular Protoporphyrin IX Following Intravestical 5-Aminolaevulinic Acid Administration: An In Vivo Study," as well as the article in *Nouvelles Dermatologiques [Dermatology News]* by P. Thomas entitled "Photothérapie

Dynamique Topique" ["Dynamic Topical Phototherapy"], the product used in treatment is ALA and not an ALA ester, which vary greatly in concentration. The ALA concentrations used are actually a minimum of 45 to 60 times higher than what is required when using an ALA ester solution (E-ALA).

Administering this substance in such strong concentrations has proven toxic to human tissue in certain instances. This toxicity, present even in the absence of light source radiation, can seriously deter generation of protoporphyrin IX (PpIX). For this reason, such concentrations either cannot be used in certain cases or are not ideal for the detection and treatment of lesions.

Furthermore, the time required to activate the active principles induced by the medicated solution is extremely long if free 5-aminolevulinic acid, that is, non-esterized ALA, is used. For this reason diagnosis and treatment using free ALA can only take place in a hospital setting, since the patient must frequently be immobilized for a very long period of time, approximately 5 hours.

In a climate where the cost of medical care is generally being reduced and preference is given to home health care, office treatment or one-day hospital care, the current treatment procedures are not only burdensome and restrictive for the patient, but costly to health insurance companies and the community.

Despite the technological progress which the use of ALA or E-ALA has contributed in terms of early diagnosis and effective treatment of certain afflictions, there are some major obstacles to its widespread use.

Description of the Invention

The goal of the present invention is to overcome these obstacles through the use of a solution designed for the diagnosis and/or treatment of cancerous lesions, particularly in the field of urology, administered in concentrations that will not prejudice biosynthesis of the active compounds and which is demonstrably very effective when applied for relatively short periods of time, making it appropriate for use in one-day clinics or even doctors' offices. Specifically, this solution must foster strong PpIX accumulation over a minimum time period and very thorough PpIX distribution throughout the treated tissue.

Sub B'

This goal is achieved using a 5-aminolevulinic acid ester (E-ALA) such as that defined in the preamble, characterized in that the concentration C of E-ALA in the solution is less than 1% and ranges form 0.01% to 0.5% ($0.01\% \leq C \leq 0.5\%$).

It has been shown in practice that use of a very low concentration of E-ALA in the solution increases PpIX synthesis and homogenizes distribution throughout the cellular layers, while at the same time greatly reducing secondary toxicity of the solution to the treated cells. This becomes even more important because when treating a tumor with dynamic phototherapy, the rapid photobleaching reduces PpIX concentration; complete destruction of the tumor implies an elevated initial accumulation of intracellular PpIX and thorough distribution throughout the layers of the tumor.

Advantageously, the ALA (E-ALA) ester producing the best results is hexylester hydrochloride ALA (h-ALA).

The solution is preferably produced by dissolving the ALA (E-ALA) ester in a solvent compatible with human or animal organisms.

Said solvent is advantageously selected from the following substances: sterilized filtered water, physiological NaCl solution, phosphate buffer solutions, with phosphate, or alcohol.

In its preferred form, the solution comprises a component for adjusting the PH to a physiological value ranging from 4.8 to 8.1.

In an advantageous form, the solution may comprise a complementary substance to prevent the transformation of the PpIX into a heme by iron complexing in the living cells.

Said complementary substance may be an EDTA (tetra acetate diaminoethyl), deferroaxmine or desferal.

Preferred Embodiment of the Invention

The present invention will be better understood with reference to the following description of a preferred embodiment of the solution according to the invention and its variations, and by way of illustration, a particularly

advantageous application of the solution in the diagnosis and/or treatment of lesions inside a cavity in a human or animal organism, such as the bladder.

A 5-aminolevulinic acid solution (E-ALA) is prepared by dissolving said substance, which may be an amorphous powder or in crystalline form, in an appropriate solvent compatible with *in vivo* use. By way of example, this solution may consist of sterilized demineralized water, physiological NaCl solution containing approximately 9% NaCl, a phosphate buffer solution, an alcohol, or a solution containing alcohol or the like.

This solution is preferably adjusted in PH to a value termed physiological, which depends on the application and primarily on what organ is to be treated. The PH value usually ranges from 4.8 to 8.1. If there is to be a procedure involving the bladder, the PH preferably ranges from 5.3 to 7.4.

The solution can be completed by the addition of a complementary substance to prevent the PpIX into from transforming into a heme by iron complexing in the living cells. This complementary substance may be an EDTA (tetra acetate diaminoethyl), deferroxamine or desferal.

One especially interesting application is the diagnosis and treatment of cancerous lesions in the field of urology, particularly on the interior bladder walls.

According to one application, the solution may be administered topically, contacting the interior walls of the organ. The bladder is filled with about 50 ml of low concentration ALA (E-ALA) ester or ALA (h-ALA) hexylester solution, e.g., a concentration C (by weight) ranging from 0.01% and 0.5% and preferably equal to 0.2%.

Instillation may last from ½ hour to 7 hours, but preferably ranges from ½ hour to 4 hours.

Surprisingly, it has been noted that with the use of these low concentrations which differ considerably from the 15 to 30% concentrations currently used in this field, the ALA (E-ALA) ester is more effective, as measured by an increased presence of fluorescent protoporphyrin IX (PpIX) apparent at the location of the lesions on the interior bladder walls and improved protoporphyrin distribution in the cell layers. Furthermore, due to these low concentrations,

cytotoxicity is reduced, which considerably decreases the risk of undesirable secondary effects. In particular, this reduced cytotoxicity favors the generation of the light sensitive and/or fluorescent substances to which free E-ALA or ALA are the precursors. Moreover, generating maximum PpIX shortens the time elapsing between administering the solution and performing the actual intervention.

One variation in application is defined as "fractionated topical method." It may comprise the following steps:

- a first bladder instillation lasting from ½ hour to 3 hours, and preferably lasting for about 2 hours;
- rinsing the bladder;
- a second instillation lasting from ½ hour to 3 hours, and preferably lasting for about 2 hours;
- rinsing the bladder.

After a waiting period of from 0 to 4 hours, and preferably for about 2 hours, fluorescent treatment and detection of the bladder can take place.

Topical solution of the ALA (E-ALA) ester solution or the ALA (h-ALA) hexylester solution may also be replaced by systemic application. In this case, the solution is administered either orally or parenterally either alone or in combination with compounds known as transporters, such as, for example, dimethylsulfoxide, glycine or the like, to enhance absorption and/or migration of the active substance, with the occurrence of the ALA (E-ALA) ester or the ALA (h-ALA) hexylester through the tissues and/or cells.

Finally, a way to activate penetration of the ALA (E-ALA) ester or the ALA (h-ALA) hexylester into the tissue or cells may consist of forming an iontophoresis on the walls of the organ concerned.

These phases are followed by one or more phototherapy and/or fluorescent treatment phases.

During phototherapy treatment, the walls of the organ concerned (for example, the bladder) are irradiated with a light beam called the excitant light, which may or may not be monochromatic, either continuously or sequentially,

preferably situated in the spectrum domain ranging from 300 to 900 nanometers and preferably between 350 and 650 nanometers.

During phototherapy proceedings the lighting E applied to the bladder walls, which is light power per surface unit, ranges from 0.1 mW/cm^2 to $1W/cm^2$, and preferably between $5mW/cm^2$ and $500mW/cm^2$. This light induces a phototoxic reaction due to the presence of protoporphyrin IX (PpIX) in particular and/or its photo-products in the tissue. The light doses may be applied homogeneously over the entire wall of the organ, or selectively at only the locations that have been identified as having lesions.

During fluorescent diagnosis, the bladder walls are irradiated using a beam with a spectral width ranging from 300 to 700 nanometers, and preferably from 350 to 650 nanometers. For these fluorescent diagnoses, the lighting E applied to the bladder walls (light power per surface unit) ranges from $1mW/cm^2$ and $1mW/cm^2$ and preferably between $50mW/cm^2$ to $500mW/cm^2$. The excitant light induces fluorescence in the substances to which E-ALA and especially h-ALA are precursors, particularly PpIX. This fluorescence is collected by an optical system and detected visually or by a specific, linear or matric detector such as a camera.

In addition to the advantages outlined above, the use of solutions with low ALA ester concentrations provides an inexpensive product for use in either phototherapy treatment or photodetection, at low production cost and with simplified Galenic pharmaceuticals.